Myocardial Reperfusion Injury

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Does Lethal Myocardial Reperfusion Injury Exist?

There are now hundreds of studies in the literature, encompassing many species, in which an intervention has been given at the end of myocardial ischemia, just before reperfusion, and has produced a decrease in the extent of myocardial infarction measured hours to days later. The evidence from these intervention studies for the existence of lethal reperfusion injury is certainly convincing to me, and I would think nearly overwhelming in the court of scientific judgment. It seems almost disingenuous to reject each and every one of these studies on the basis of some technical point or presumed mismatching of animals in treated and control groups.

So why is there continued controversy? One reason is that lethal reperfusion injury is difficult to prove directly. One needs to show that the very same region of myocardium was viable at the end of ischemia and undergoes irreversible injury after reperfusion. Furthermore, it is important to show that myocyte death by reperfusion is not simply the consequence of an inexorable series of cellular events set in motion during ischemia and playing itself out over the next several hours during reperfusion. Apoptosis of myocytes might represent this type of phenomenon. Apoptosis has been shown to exist following ischemia-reperfusion, but evidence suggests that it is the oxygen radicals generated during reperfusion that trigger apoptosis [1,2]. The best evidence against myocyte death during reperfusion representing exclusively an ischemia-triggered phenomenon is the ability of interventions given just before reperfusion to limit infarct size. If death were a foregone conclusion, myocyte preservation by a treatment operative during reperfusion would not be possible.

Our laboratory has completed two studies that directly support the existence of lethal reperfusion injury. Electron microscopy performed by Jutta Schaper on multiple tissue samples from dogs with 90 minutes of ischemia and 0–180 minutes of reperfusion showed that the frequency of irreversible injury was independently related to both the severity of ischemia and the duration of reperfusion [3]. Samples with collateral flow <0.05 ml/min/g had extensive ischemic injury, but no additional injury during reperfusion. Samples with greater collateral flow had more extensive irreversible injury at 180 minutes reperfusion than was predicted by the severity of ischemia. In another study, the uptake of radionuclide labeled 2-deoxyglucose was used as a marker of regional myocardial viability early after reperfusion and was compared with the development of necrosis in the same region of myocardium 4 hours later [4]. Many tissue samples taken from the TTC-negative necrotic area had preserved 2-deoxyglucose uptake, indicating that they were viable early after reperfusion but suffered subsequent irreversible injury. When collateral flow was <4% of non-ischemic flow, infarcts were large and almost all of the necrosis occurred during ischemia. When collateral flow was 4–10% of normal, most of the necrosis occurred during reperfusion. When collateral flow was >10% of normal, infarcts were relatively small and necrosis occurred virtually entirely during reperfusion. The amount of necrosis was maximal when collateral flow was about 5% of normal: Reperfusion necrosis comprised up to 40% of the risk region and about 50% of the total infarct size. This is similar to the 30–50% reduction in infarct size reported in most intervention studies aimed at eliminating reperfusion injury.

A second reason for continued controversy is related to the inconsistent effects of interventions. I believe this is due, in part, to a lack of appreciation of the importance of collateral flow in determining whether or not reperfusion injury occurs. Inconsistent results also stem from inadequate knowledge about the biology of reperfusion injury, including the time course, mechanisms, and modifying influences. A negative study does not necessarily mean that lethal reperfusion injury is absent; it may merely reflect our inability to recognize and control certain critical experimental variables. In addition, most negative studies in the literature do not have the statistical power to conclude with any confidence that the intervention is truly ineffective.

When and how does lethal reperfusion injury occur? Although some of the more obvious pathological changes in reperfused myocardium occur within minutes of reperfusion, it is not clear that this is when most lethal reperfusion injury actually occurs. At-
tempts to prevent explosive cell swelling, intracellular calcium entry, or calcium-activated cellular processes (e.g., protease activity or myofibrillar contraction) have been marginally effective in vivo animal models. Results in buffer or blood-perfused isolated hearts, often under nonworking conditions, are difficult to extrapolate to the in vivo situation. Although an ischemic myocyte undergoing explosive swelling, calcium overload, or contracture with reperfusion could conceivably have been injured reversibly before reperfusion, it seems more likely that the cell was already dead or dying, and because of this, was unable to regulate cell volume or intracellular calcium concentration during reperfusion.

Interventions inhibiting postischemic inflammation have successfully reduced infarct size in many studies across a wide variety of species. Approaches have included mechanical removal of neutrophils [5]; generalized suppression of neutrophil function; prevention of neutrophil activation by blocking the action of complement, leukotrienes, platelet activating factor, cytokines, and chemokines; prevention of neutrophil adhesion by antibodies against endothelial or neutrophil adhesion molecules [6,7]; administration of perfluorochemicals, adenosine, or nitric oxide donors to suppress neutrophil adhesion and function, and oxygen radical scavengers to detoxify neutrophil-generated oxidant species [8]. The body of evidence supporting antineutrophil interventions for limiting reperfusion injury is both strong and consistent.

Ischemia/reperfusion triggers changes in vascular endothelial cells, possibly through processes initiated by oxygen radicals, resulting in new expression of adhesion molecules (P-selectin and ICAM-1), generation of neutrophil activators (e.g., PAF), diminished release of nitric oxide, and changes in the actin cytoskeleton that allow greater vascular permeability. All of these endothelial events, in addition to the release of neutrophil chemottractants and activators from other components of reperfused myocardium, promote neutrophil targeting, endothelial attachment, transendothelial migration, and adhesion-related oxidant release [9]. The latter appears to represent the final common pathway for neutrophil-mediated myocyte killing. Neutrophil influx into reperfused myocardium occurs over several hours, consistent with our observation that lethal reperfusion injury takes over 2 hours to develop [3]. It is unknown how long these processes may occur or when evolution of a reperfused acute infarct can be said to be “completed.”

**Is Reperfusion Injury Clinically Important?**

In my opinion, lethal reperfusion injury has been convincingly demonstrated in many animal models. Our own data indicate that about 50% of final infarct size in dogs with 90 minutes of coronary artery occlusion is caused by reperfusion injury, although this value is probably less in dogs with very severe ischemia and greater in those with less severe ischemia [4,7]. It is considerably more difficult to measure risk region, collateral blood flow, duration of coronary occlusion, infarct size, and the extent of reperfusion injury in patients with acute infarction. Progress may occur through application of modern technology, including multilead electrocardiographic monitoring, to determine the time of arterial patency and the presence of reocclusions, radionuclide perfusion imaging to measure collateral flow and risk region size, and proton magnetic resonance imaging with gadolinium contrast enhancement to measure infarct size, the extent of no reflow, and the amount of residual functioning myocardium [10].

Despite the difficulties with making measurements in patients, there is no reason to think that the fundamental biology of ischemia/reperfusion is substantially different between humans and experimental animals. All species examined produce oxygen radicals in reperfused tissue, activate complement, express adhesion molecules, produce proinflammatory cytokines, and accumulate neutrophils and monocytes. These processes occur in all organs examined, including the heart. The only question is whether the conditions that are present in patients with reperfused acute myocardial infarction, or other ischemia/reperfusion syndromes, such as unstable angina, cardiac surgery, cardiac transplantation, or cardiopulmonary resuscitation, promote the occurrence of postischemic inflammation and associated reperfusion injury. Ischemia must be severe enough to initiate inflammation during reperfusion, but not severe enough to itself lethally injure most or all of the myocardium at risk.

Generally speaking, reperfusion salvages myocardium by relieving ischemia. Reperfusion injury reduces the amount of benefit that can be achieved by reperfusion. Reperfusion injury does not extend necrosis beyond the area that would undergo infarction without reperfusion.

**Should Interventions Be Designed and Marketed to Attenuate Reperfusion Injury?**

I believe that reperfusion injury of the heart, as well as of other critical organs, such as the brain, kidneys, and intestines, is an important clinical problem that deserves the efforts of investigators in academia and pharmaceutical and biotechnology companies to design and implement safe and effective therapies. The development of such therapies depends on a proper understanding of the pathobiology involved. Inflammation appears to represent an important mechanism for reperfusion injury, and presents numerous potential therapeutic targets, including adhesion molecules, proinflammatory activators, and oxygen radicals. The